Journal of Physiology (1989), **410**, *pp.* 341–350
With 5 text-figures
Printed in Great Britain

A COMPARISON OF HEAD-DOWN TILT WITH LOW-DOSE INFUSION OF ATRIAL NATRIURETIC PEPTIDE IN MAN

BY MICHAEL J. ALLEN*, VINCENT T. Y. ANG† AND DAVID BENNETT*
*From the Departments of Medicine *One and †Two, St George's Hospital Medical School, London SW17 0RE*

(*Received 1 June 1988*)

SUMMARY

1. Procedures that increase atrial pressure, such as head-down tilt, result in an increase in plasma atrial natriuretic peptide (ANP) and a natriuresis, but a direct cause-and-effect relationship between these two responses has not been established. This study was undertaken to compare the effects of head-down tilt with exogenous ANP on renal function.

2. Eight normal sodium-replete volunteers underwent a 3 h placebo infusion, a 3 h ANP infusion at 1·2 pmol kg^{-1} min^{-1} and a 3 h period of head-down tilt. Each procedure was performed on a separate day, in random order.

3. ANP and head-down tilt produced similar increases in sodium excretion (65 ± 24 and 68 ± 16%, respectively). ANP did not increase urine flow significantly more than placebo. Head-down tilt increased urine flow significantly more than placebo and ANP.

4. Plasma ANP rose from $8{\cdot}1 \pm 1{\cdot}0$ to $11{\cdot}4 \pm 2{\cdot}5$ pg ml^{-1} during head-down tilt and from $6{\cdot}5 \pm 1{\cdot}4$ to $32{\cdot}3 \pm 10{\cdot}7$ pg ml^{-1} with ANP infusion.

5. ANP infusion had no significant effects on systemic haemodynamics whilst head-down tilt increased cardiac output and reduced heart rate and an index of systemic vascular resistance.

6. ANP infusion, whilst achieving a natriuretic response similar to that of tilt, was associated with a 3-fold higher mean plasma ANP level. Although plasma ANP rose during both ANP infusion and tilt, there was a lack of correlation between natriuretic response and plasma ANP.

7. The results are not compatible with a direct cause-and-effect relationship between plasma ANP and sodium excretion during head-down tilt.

INTRODUCTION

Manoeuvres which increase central blood volume (lower body positive pressure, water immersion, supine posture) will produce both a rise in ANP and a natriuretic response (Epstein, 1978; Bennett, Tighe & Wegg, 1982; Solomon, Atherton, Bobinski & Green, 1986; Anderson, Millar, O'Hare, Mackenzie, Corrall & Bloom, 1986). Although increases in central blood volume produce a number of hormonal and neural reactions it has been suggested that ANP is the primary mediator of

these responses. Blocking of the natriuretic response to volume expansion by monoclonal antibodies to ANP provides evidence to support this suggestion (Hirth, Stasch, John, Kazda, Morich, Neuser & Wohlfeil, 1986). On the other hand cardiac denervation, both in an animal model (Goetz, Wang, Geer, Leadley & Reinhardt, 1986; Knapp, Hicks, Linden & Mary, 1986) and in human cardiac transplant recipients (Wilkins, Gammage, Lewis, Bun Tan & Weissberg, 1988), has been shown to antagonize the natriuresis associated with atrial stretch but not to affect the ANP release, suggesting a dissociation between these two responses.

To investigate further the relationship between atrial distension, ANP and natriuresis we have compared an infusion of ANP at 1·2 pmol kg^{-1} min^{-1} with a placebo infusion and with the effects of 10 deg head-down tilt. An ANP infusion at this rate will produce a natriuresis and is said to increase plasma ANP within the physiological range (Anderson, Donckier, Payne, Beacham, Slater & Bloom, 1987). We set out to examine the renal effects, the effects on plasma volume as reflected in the haematocrit and the haemodynamic effects as assessed by changes in blood pressure, heart rate and Doppler measurements of ascending aortic blood flow.

METHODS

Eight normal volunteers were recruited and fully informed written consent was obtained. Approval for the study was given by the District Medical Ethical Committee. Volunteers were asked to eat their normal sodium diet supplemented with slow sodium (1·2 g 8 hourly) for 3 days. Caffeine and alcohol were prohibited for 24 h prior to the study. Subjects took 500 mg lithium carbonate at 22.00 h the night before each study and were fasted on the day of the study except for 250 ml water at 08.00 h and 250 ml water on arrival in the lab at 09.00 h.

Each subject attended three times with a minimum of 3 days between visits. On each visit the first hour was spent as a control period sitting upright. Subjects then underwent one of three manoeuvres for the next 3 h. The manoeuvres were an ANP infusion (1·2 pmol kg^{-1} min^{-1}), a placebo infusion and a period of head-down tilt. Each subject underwent a different manoeuvre on each visit, given in random order. The infusions were given in the sitting position. α-Human ANP (Penninsula Laboratories, St Helens, UK) was passed through a 2 μm filter and diluted in 0·9% saline with 0·45% human albumin solution (Blood Products Laboratory, Elstree, UK). Placebo consisted of carrier alone. The total volume of each infusion was 60 ml which was given at 20 ml h^{-1} by infusion pump (Vickers Medical). After the first hour, fluid replacement was given orally as tap water in a volume equal to the previous hour's urine output plus 30 ml up to a maximum of 500 ml h^{-1}.

Blood samples were taken at the start of the study for determination of plasma sodium (P_{Na}) and creatinine (P_{Cr}) and hourly for haematocrit estimation and serum lithium assay. Blood was also taken after 30 and 60 min of the control period and hourly thereafter into chilled 10 ml polypropylene tubes containing ethylenediamine acetate and aprotinin for ANP assay. The samples for ANP were immediately centrifuged, the plasma separated, frozen on dry ice and stored at −20 °C until assayed.

Volunteers stood hourly to void urine for volume, sodium, creatinine and lithium assays. From these variables calculations were made of flow rate, sodium excretion rate, creatinine clearance (C_{Cr}), fractional sodium excretion, using C_{Cr} as the measure of glomerular filtration rate, clearance of sodium (C_{Na}), the clearance of sodium by the proximal nephron which is equivalent to the clearance of lithium (C_{Li}) (Thomsen, 1984) and the fractional excretion of sodium by the distal nephron ($C_{Na}/C_{Li} \times 100$).

Plasma sodium was measured by an ion-selective electrode, plasma and urinary creatinine by the Jaffe method, urinary sodium by flame photometry and plasma and urinary lithium by atomic absorption spectrometry. Haematocrit was estimated as the mean of two samples measured using a micro-haematocrit centrifuge. Plasma for ANP assay was separated on a Sep-Pak C18 column and assayed as previously described (Allen, Ang, Bennett & Jenkins, 1988).

Haemodynamic measurements were made at the end of the control period and at 15, 30, 60, 120 and 180 min following the start of infusion or head-down tilt. Blood pressure was measured by sphygmomanometry taking the diastolic as the fourth Korotkoff phase and ascending aortic blood flow by Doppler (Exerdop, Quinton, Seattle, USA). Doppler provides a variable known as stroke distance (SD) which is a linear index of stroke volume. Minute distance (MD) is calculated as the product of SD and heart rate, and is a linear index of cardiac output. If multiplied by the aortic cross-sectional area these two indices (SD and MD) would be equal to the stroke volume and cardiac output. Mean arterial blood pressure (diastolic + 1/3 pulse pressure) divided by MD was used as an index of systemic vascular resistance (ISVR).

Statistics

Results are expressed as mean ± S.E.M. Analysis of variance was used throughout except for the C_{Li} and C_{Na}/C_{Li} data where Student's paired t tests were used to compare each measurement with both the control period and the placebo effect.

RESULTS

Renal effects

Sodium excretion was not significantly affected by the placebo infusion (230 ± 29 to 202 ± 34 μmol min^{-1}), was increased by ANP infusion from 188 ± 44 to 257 ± 37 μg min^{-1} and was increased by head-down tilt from 202 ± 30 to 310 ± 28 μmol min^{-1}. Because of differing baseline levels these data were transformed into percentage changes in order to compare both tilt and ANP infusion data with placebo data. Placebo infusion did not produce a significant effect. ANP infusion and the period of head-down tilt produced increases in sodium excretion of 65 ± 24 and 68 ± 16%, respectively, which were delayed until the second and third hours. Both of these increases were significantly different from the placebo response ($P < 0.005$) but not from each other (Fig. 1*A*).

Creatinine clearance was not significantly affected by any of the manoeuvres. Fractional excretion of sodium was unchanged with placebo but increased from 0·83 ± 0·16 to 1·12 ± 0·12% with ANP and from 0·87 ± 0·12 to 1·34 ± 0·10% with tilt (Fig. 1*B*).

Urinary flow increased significantly with placebo from a mean of 5·8 ± 1·2 ml min^{-1} during the control period to 7·9 ± 0·5 ml min^{-1}during the three hours of infusion ($P < 0.01$). The urinary flow response to ANP was similar to placebo (4·4 ± 1·9 rising to 6·4 ± 1·3 ml min^{-1}, n.s.) but there was a greater diuresis associated with head-down tilt (5·6 ± 1·2 rising to 10·8 ± 0·4 ml min^{-1}, $P < 0.001$). This diuresis began within the first hour of tilt (Fig. 2) and was significantly greater than that associated with placebo ($P < 0.001$) and that associated with ANP ($P < 0.0001$).

Although there was an increase in lithium clearance with the placebo infusion this was not significant. Lithium clearance increased with the ANP infusion from 22·0 ± 2·7 to 27·4 ± 2·0 ml min^{-1} after 1 h ($P < 0.05$) and remained elevated for the duration of the infusion. With head-down tilt there was a similar response in the first hour (24·3 ± 2·9 rising to 28·3 ± 3·8 ml min^{-1}, $P < 0.05$) but the increase was not maintained beyond the first hour (Fig. 3). The fractional excretion of sodium by the distal nephron ($FE_{Na,distal}$, $C_{Na}/C_{Li} \times 100$) was not significantly changed by placebo. With head-down tilt $FE_{Na,distal}$ initially showed a non-significant fall from 6·4 ± 1·1 to 5·8 ± 1·0% but then increased in the second and third hours to 10·9 ± 1·9%

($P < 0.05$) and $9.6 \pm 1.0\%$ ($P < 0.05$) respectively. A similar reduction was seen in the first hour of ANP infusion (7.3 ± 2.3 to $4.0 \pm 0.7\%$) and although this subsequently recovered to the control level, it did not increase beyond the control, as it did with head-down tilt.

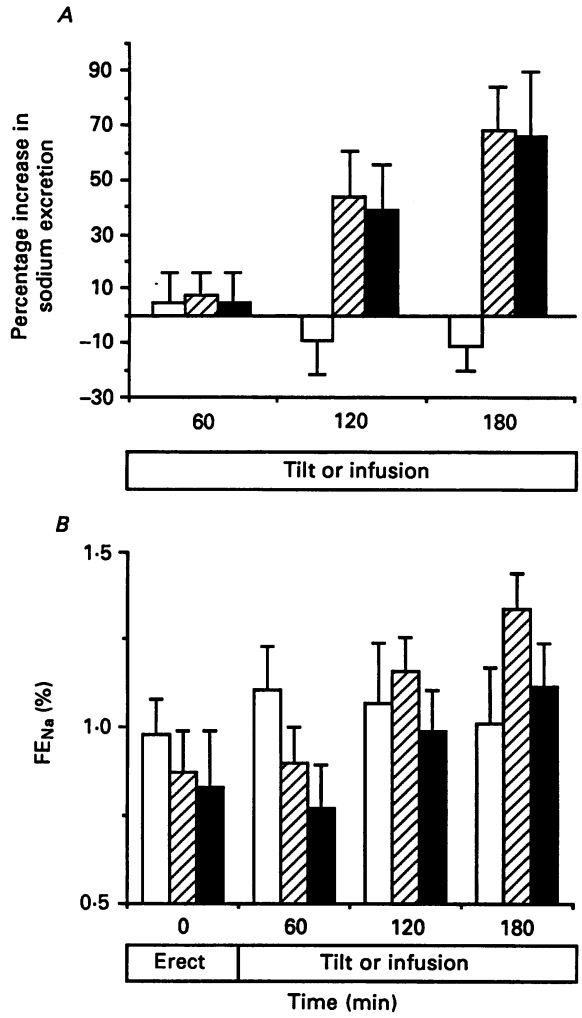

Fig. 1. Effect of placebo (□), head-down tilt (▨) and ANP (■), on percentage increase in sodium excretion (*A*) and fractional sodium excretion (*B*) (means ± S.E.M.). Analysis of variance: ANP *vs.* placebo, $P < 0.005$; tilt *vs.* placebo, $P < 0.005$; ANP *vs.* tilt, n.s.

Haemodynamic effects

Heart rate was not significantly affected by either the ANP or placebo infusions but fell from 60.8 ± 3.3 to 54.9 ± 2.5 min^{-1} ($P < 0.01$) with head-down tilt. No consistent changes were seen in arterial blood pressure in any of the groups. There was no significant difference between ANP and placebo in relation to MD or ISVR. Head-down tilt, however, increased MD from 834 ± 69 to 963 ± 74 cm and reduced

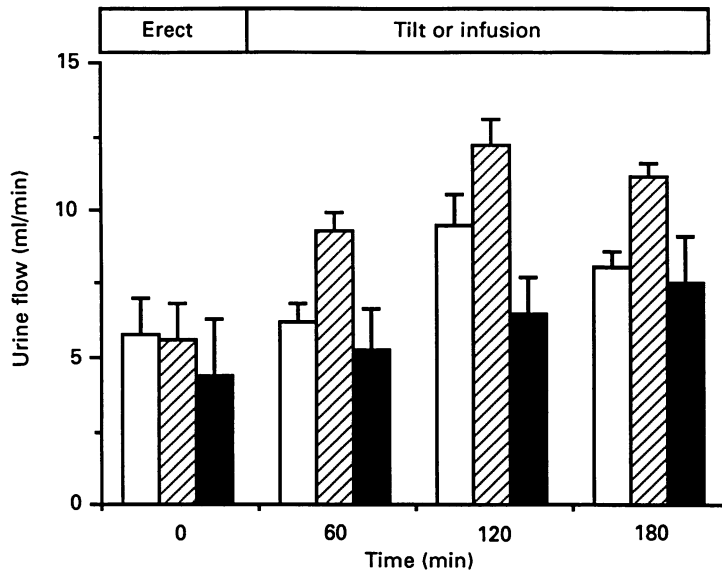

Fig. 2. Effect of placebo (□), head-down tilt (▨) and ANP (■) on urinary flow (means ± S.E.M.). Analysis of variance: ANP *vs.* placebo, n.s.; tilt *vs.* placebo, $P < 0.001$; ANP *vs.* tilt, $P < 0.0001$.

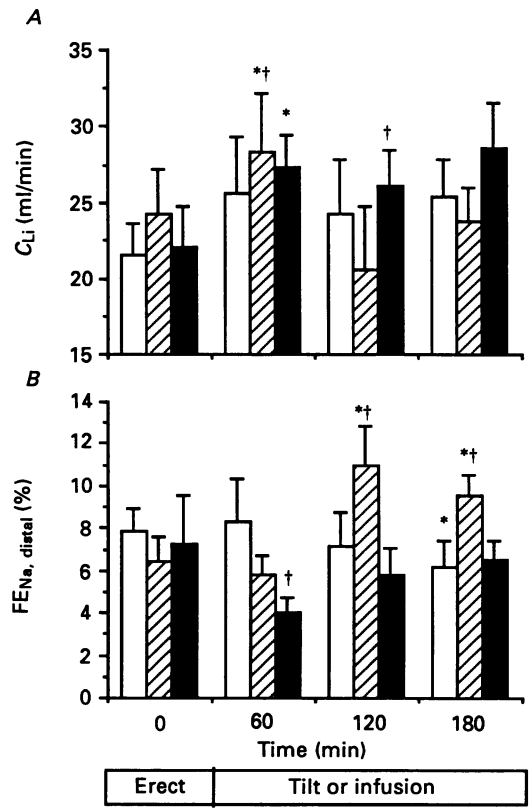

Fig. 3. Effect of placebo (□), head-down tilt (▨) and ANP (■), on clearance of lithium (*A*, C_{Li}) and fractional clearance of sodium by the distal nephron (*B*, $FE_{Na,distal}$) (means ± S.E.M.). Student's paired *t* tests: $*P < 0.05$ relative to time 0; $\dagger P < 0.05$ relative to placebo.

ISVR from $0{\cdot}121 \pm 0{\cdot}007$ to $0{\cdot}098 \pm 0{\cdot}006$ mmHg cm^{-1}. Both of these changes with head-down tilt were maintained for the duration of the study and both were significantly different from the placebo responses ($P < 0{\cdot}001$).

Other results

Venous haematocrit was unchanged by the placebo infusion ($43{\cdot}7 \pm 1{\cdot}1$ to $43{\cdot}8 \pm 1{\cdot}3$%), fell with head-down tilt ($43{\cdot}8 \pm 0{\cdot}9$ to $43{\cdot}1 \pm 0{\cdot}9$%) and rose with the ANP infusion ($43{\cdot}5 \pm 0{\cdot}5$ to $44{\cdot}3 \pm 0{\cdot}6$%). Analysis of variance shows that there were significant differences ($P < 0{\cdot}001$) with a treatment effect from ANP ($P < 0{\cdot}05$) and significant differences between ANP and head-down tilt and between head-down tilt and placebo.

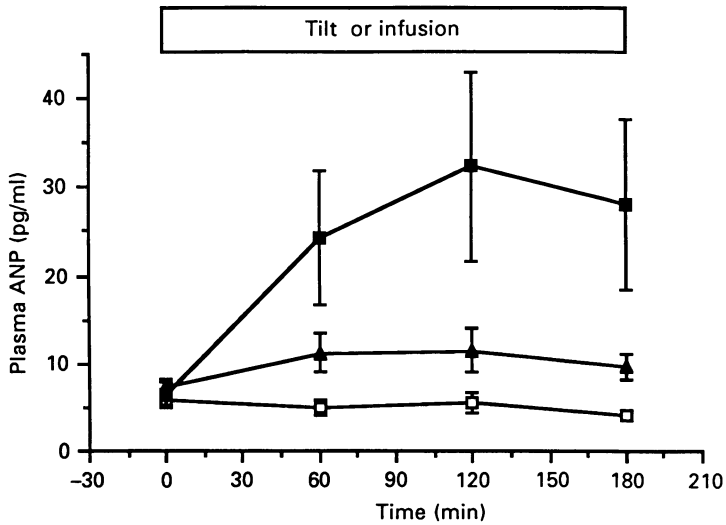

Fig. 4. Effect of placebo (□), head-down tilt (▲) and ANP (■), on plasma ANP (means ± S.E.M.). Analysis of variance: ANP *vs.* placebo, $P < 0{\cdot}001$; tilt *vs.* placebo, $P < 0{\cdot}001$; ANP *vs.* tilt, $P < 0{\cdot}01$.

Plasma ANP did not change with placebo infusion ($5{\cdot}7 \pm 0{\cdot}5$ pg ml^{-1} at baseline, $4{\cdot}2 \pm 0{\cdot}5$ pg ml^{-1} at completion). With head-down tilt plasma ANP rose from $8{\cdot}1 \pm 1{\cdot}0$ pg ml^{-1} to a peak of $11{\cdot}4 \pm 2{\cdot}5$ pg ml^{-1} which was significantly different from the placebo response ($P < 0{\cdot}001$). The ANP infusion produced an increase in plasma ANP from $6{\cdot}5 \pm 1{\cdot}4$ to $32{\cdot}3 \pm 10{\cdot}7$ pg ml^{-1} which was significantly different from both the placebo response ($P < 0{\cdot}001$) and the response to head-down tilt ($P < 0{\cdot}01$, Fig. 4).

During infusion of ANP, plasma levels varied between individuals from 11·7 to 86·6 pg ml^{-1}. The natriuretic response did not correlate with plasma ANP (Fig. 5) or any of the haemodynamic variables but did correlate with changes in creatinine clearance ($r = 0{\cdot}68$, $P < 0{\cdot}005$) and haematocrit ($r = 0{\cdot}45$, $P < 0{\cdot}05$).

During head-down tilt plasma ANP varied between individuals from 2·8 to 21·8 pg ml^{-1}. Again the natriuretic response did not correlate with changes in plasma ANP (Fig. 5) but there were close positive correlations with ISVR ($r = 0{\cdot}74$, $P < 0{\cdot}003$) and systolic blood pressure ($r = 0{\cdot}64$, $P < 0{\cdot}02$).

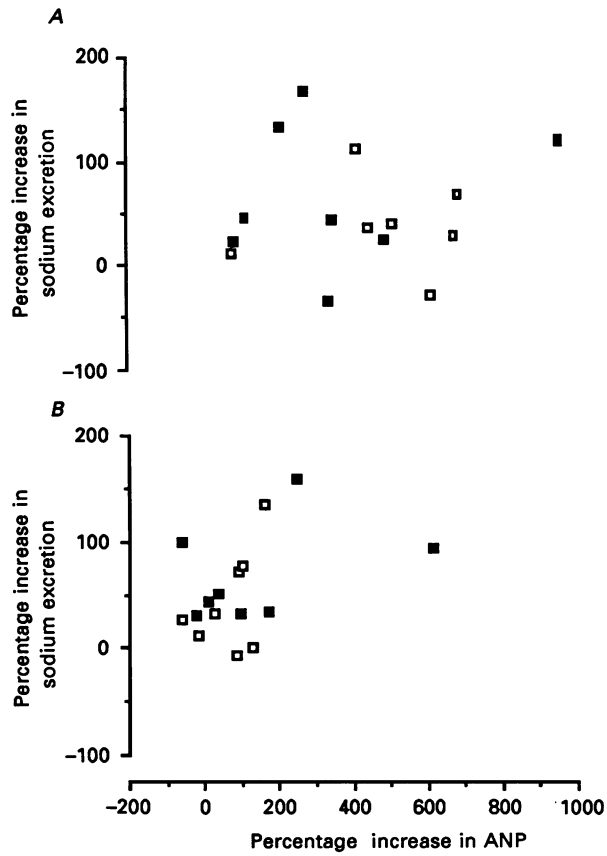

Fig. 5. Lack of correlation between the percentage increase in sodium excretion and the percentage increase in plasma ANP during head-down tilt (*A*) and during ANP infusion (*B*) in eight volunteers. Results are given for the second (□) and third (■) hours of tilt or ANP infusion.

DISCUSSION

This study demonstrates that although ANP infusion at this rate and head-down tilt produced similar natriuretic responses, this was only achieved by ANP infusion with a three-fold higher mean plasma ANP concentration. There was no correlation between the increase in sodium excretion and the increase in plasma ANP associated with either head-down tilt or ANP infusion (Fig. 5).

Hirth *et al.* (1986) have provided evidence suggesting that ANP is of primary importance in the natriuresis of volume expansion. They have shown, in rats, that the renal response to acute volume expansion, with homologous blood transfusion, can be blocked by the addition of monoclonal antibodies to ANP. In contrast to this it has been shown that cardiac denervation antagonizes the natriuretic response to atrial distention but does not inhibit ANP release, suggesting a dissociation between the two responses (Goetz *et al.* 1986; Knapp *et al.* 1986). One way of reconciling these two studies would be if it could be demonstrated that cardiac denervation removes

a factor on which the renal action of ANP is dependent. Such a factor might be the changes in systemic haemodynamics associated with atrial distention or the inactivation of renal sympathetic nerve traffic. There is circumstantial evidence for the latter in that afferent pathways exist both in cardiopulmonary sympathetic nerves (Weaver, 1977; Niitani, Tomomatsu, Ohba, Yoshida & Yagi, 1988) and in the vagi (Karim, Kidd, Malpus & Penna, 1972) which modify renal sympathetic nerve activity (RSNA) and increased RSNA has been shown to inhibit the renal response to both volume expansion (Gill & Casper, 1969) and ANP (Koepke & DiBona, 1987*a*; Koepke, Jones & DiBona, 1987*b*; Torikai, 1988).

In this study an ANP infusion producing plasma levels of the same order of magnitude as the physiological range caused a modest increase in haematocrit but no significant haemodynamic changes. On the other hand head-down tilt tended to lower haematocrit, to increase cardiac output (10%) and to reduce an index of systemic vascular resistance (24%). One would not expect changes in cardiac output, within this range, to affect sodium excretion but the changes seen in systemic vascular resistance may also reflect changes in renal sympathetic nerve activity and, as discussed above, this may facilitate the renal response to ANP.

During tilt the degree of natriuresis showed a close positive correlation with ISVR and systolic blood pressure. In other words, although a reduction of sympathetic activity to the kidney may enhance the response to ANP, if systemic sympatholytic effects are profound enough to reduce renal perfusion pressure by a steal effect, natriuresis may be impaired. Dependence of the renal response to volume expansion on both blood pressure (Schrier, McDonald, Jagger & Lauler, 1967) and renal perfusion pressure (McDonald & deWardener, 1965) is well recognized.

During infusion of ANP the natriuretic response did not correlate with any of the haemodynamic parameters but did correlate with changes in creatinine clearance and changes in haematocrit. These correlations probably reflect the level of biological activity of ANP rather than indicating mechanisms of natriuresis.

The highly variable plasma levels achieved during infusion must reflect varying clearances of the peptide between individuals. The biological activity of ANP probably relates not only to the plasma level but also the rate at which it is cleared from the circulation either by proteases or by binding to specific receptors.

The effects of ANP and head-down tilt on lithium clearance (Fig. 3) are broadly similar with an immediate increase in proximal sodium clearance. This increase is, however, better maintained with ANP than with head-down tilt. In the first hour this increased proximal clearance is compensated for, in both groups, by a reduction in fractional distal nephron sodium clearance. Subsequently distal nephron clearance increases giving rise to a net increase in sodium loss in the urine. This delayed distal nephron response is much more marked with head-down tilt than ANP alone and may therefore reflect an additional natriuretic mechanism, perhaps aldosterone suppression or the release of a Na^+,K^+-ATPase inhibitor.

These results also demonstrate that head-down tilt is a more potent stimulus to urinary flow than ANP alone suggesting that, during volume expansion, any contribution that ANP makes to the suppression of arginine vasopressin (Allen *et al.* 1988) is overshadowed by more direct effects.

In conclusion this study suggests that the renal response to ANP alone is not as

marked as that to central blood volume expansion from head-down tilt. This may be due to the presence of other independent and more potent natriuretic mechanisms or may indicate dependence of ANP on other mechanisms. Maintenance of renal perfusion pressure and/or a reduction in renal sympathetic tone may be important in this respect.

We thank Dr G. E. Levin for his guidance with lithium assays. Financial assistance was provided by the Special Trustees of St George's Hospital.

REFERENCES

ALLEN, M. J., ANG, V. T. Y., BENNETT, E. D. & JENKINS, J. S. (1988). Atrial natriuretic peptide inhibits osmolality induced arginine vasopressin release in man. *Clinical Science* **75**, 35–39.

ANDERSON, J. V., DONCKIER, J., PAYNE, N. N., BEACHAM, J., SLATER, J. D. H. & BLOOM, S. R. (1987). Atrial natriuretic peptide: evidence of action as a natriuretic hormone at physiological plasma concentrations in man. *Clinical Science* **72**, 305–312.

ANDERSON, J. V., MILLAR, N. D., O'HARE, J. P., MACKENZIE, J. C., CORRALL, R. J. M. & BLOOM, S. R. (1986). Atrial natriuretic peptide: physiological release associated with natriuresis during water immersion in man. *Clinical Science* **71**, 319–322.

BENNETT, E. D., TIGHE, D. & WEGG, W. (1982). Abolition by dopamine blockade of the natriuretic response produced by lower body positive pressure. *Clinical Science* **63**, 361–366.

EPSTEIN, M. (1978). Renal effects of head-out water immersion in man: implications for an understanding of volume homeostasis. *Physiological Reviews* **58**, 529–581.

GILL, J. R. & CASPER, A. G. (1969). Role of sympathetic nervous system in the renal response to haemorrhage. *Journal of Clinical Investigation* **48**, 915–922.

GOETZ, K. L., WANG, B. C., GEER, P. G., LEADLEY, R. J. & REINHARDT, H. W. (1986). Atrial stretch increases sodium excretion independently of release of atrial peptides. *American Journal of Physiology* **250**, R946–950.

HIRTH, C., STASCH, J. P., JOHN, A., KAZDA, S., MORICH, F., NEUSER, D. & WOHLFEIL, S. (1986). The renal response to acute hypervolaemia is caused by atrial natriuretic peptides. *Journal of Cardiovascular Pharmacology* **8**, 268–275.

KARIM, F., KIDD, C., MALPUS, C. M. & PENNA, P. E. (1972). The effects of stimulation of the left atrial receptors on sympathetic efferent nerve activity. *Journal of Physiology* **227**, 243–260.

KNAPP, M. F., HICKS, M. N., LINDEN, R. J. & MARY, C. A. S. G. (1986). Evidence against ANP as a natriuretic hormone during atrial distension. *Journal of Endocrinology* **109**, R5–8.

KOEPKE, J. P. & DIBONA, G. F. (1987*a*). Blunted natriuresis to atrial natriuretic peptide in chronic sodium-retaining disorders. *American Journal of Physiology* **252**, F865–871.

KOEPKE, J. P., JONES, S. & DIBONA, G. F. (1987*b*). Renal nerves mediate blunted natriuresis to atrial natriuretic peptide in cirrhotic rats. *American Journal of Physiology* **252**, R1019–1023.

MCDONALD, S. J. & DEWARDENER, H. E. (1965). The relationship between the renal arterial perfusion pressure and the increase in sodium excretion which occurs during an infusion of saline. *Nephron* **2**, 1–14.

NIITANI, S., TOMOMATSU, E., OHBA, H., YOSHIDA, Y. & YAGI, S. (1988). Renal nerve and cardiovascular responses to cardiac receptor stimulation in rabbits. *American Journal of Physiology* **254**, R192–196.

SCHRIER, R. W., MCDONALD, K. M., JAGGER, P. I. & LAULER, D. P. (1967). The role of the adrenergic nervous system in the renal response to acute extracellular fluid volume expansion. *Proceedings of the Society for Experimental Biology and Medicine* **125**, 1157–1162.

SOLOMON, L. R., ATHERTON, J. C., BOBINSKI, H. & GREEN, R. (1986). Effect of posture on plasma immunoreactive atrial natriuretic concentrations in man. *Clinical Science* **71**, 299–305.

THOMSEN, K. (1984). Lithium clearance: a new method for determining proximal and distal tubular reabsorption of sodium and water. *Nephron* **37**, 217–223.

TORIKAI, S. (1988). A renorenal suppression in response to atrial natriuretic peptide in the unilateral ischaemic rat. *Clinical Science* **74**, 519–525.

WEAVER, L. C. (1977). Cardiopulmonary sympathetic afferent influences on renal nerve activity. *American Journal of Physiology* **233**, H592–599.

WILKINS, M. R., GAMMAGE, M. D., LEWIS, H. M., BUN TAN, L. & WEISSBERG, P. L. (1988). Effect of lower body positive pressure on blood pressure, plasma atrial natriuretic factor concentration, and sodium and water excretion in healthy volunteers and cardiac transplant recipients. *Cardiovascular Research* **22**, 231–235.